



UNIVERSITI PUTRA MALAYSIA

**ROLES OF TRANSFORMING GROWTH FACTOR-BETA,
INSULIN-LIKE GROWTH FACTORS AND PROTEASES
IN HUMAN BREAST CANCER**

WONG SHEW FUNG

FPSK (M) 2002 6

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By

WONG SHEW FUNG

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia
in Fulfilment of the Requirements for the Degree of Master of Science**

July 2002



Specially dedicated to,

My beloved parents, husband, brothers, supervisors and friends

For their invaluable support, love, patience and intellectual stimulation.....

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirements for the degree of Master of Science

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By

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July 2002

Chairman : Associate Professor Dr Seow Heng Fong

Faculty : Medicine and Health Sciences

Transforming growth factor-beta ($\text{TGF}\beta$) is present in breast cysts, normal and cancerous breast tissue. It plays an important role in oestrogen metabolism. However, $\text{TGF}\beta$ is present predominantly in latent forms and the mechanisms by which latent $\text{TGF}\beta$ is activated physiologically remain largely an enigma.

The aims of this study were:-

- 1) to investigate the effects of $\text{TGF}\beta_1$, IGF-I and IGF-II on cell growth, E_1 STS and E_2 DH activities in the hormone-dependent MCF-7 and hormone-independent MDA-MB-231 human breast cancer cell lines.
- 2) to investigate the effect of Cathepsin D and PSA on $\text{TGF}\beta_1$ and $\text{TGF}\beta_2$ mRNA expression, and their protein levels in both of the cell lines.
- 3) to study the effect of Cathepsin D and PSA on Type 1 and Type 2 E_2 DH mRNA expression, E_1 and E_2 levels in both cell lines.

TGF β_1 , IGF-I and IGF-II either alone or in combination inhibited cell growth of both cell lines but no additive or synergistic effects were observed. These treatments significantly stimulated E₁STS activity in the MCF-7 cell line except for TGF β_1 alone and TGF β_1 and IGF-I in combination. Only TGF β_1 and IGF-II acted synergistically to stimulate E₁STS activity in the MCF-7 cells. There was no significant effect on E₁STS activity in the MDA-MB-231 cells with any of the treatments. In the MCF-7 cell line, TGF β_1 and IGF-I, IGF-I and IGF-II, and TGF β_1 , IGF-I and IGF-II acted synergistically to stimulate reductive E₂DH activity while only TGF β_1 , IGF-I and IGF-II synergistically stimulated oxidative E₂DH activity. There were no synergistic effects on both oxidative and reductive E₂DH activities in the MDA-MB-231 cell line.

Cathepsin D (200 ng/ml) induced TGF β_1 mRNA at 72 hours whilst Cathepsin D concentrations of 50, 100 and 200 ng/ml reduced TGF β_2 mRNA at 8 hours in the MCF-7 cell line. PSA (50 ng/ml) downregulated TGF β_1 and TGF β_2 mRNA at 72 and 8 hours, respectively. Cathepsin D (100 ng/ml) upregulated TGF β_2 mRNA at 24 hours in the MDA-MB-231 cell line. PSA (50, 200 and 400 ng/ml) induced TGF β_1 mRNA at 24 hours in the MDA-MB-231 cells. Both Cathepsin D and PSA were unable to activate latent TGF β_1 and TGF β_2 in both cell lines while a significant increase of active TGF β_2 throughout the experiment was observed. Cathepsin D and PSA had no effect on reductive and oxidative E₂DH mRNA in the MCF-7 cell line. Cathepsin D (50, 100 and 200 ng/ml) at 24 hours and 50 ng/ml at 4 hours upregulated reductive and oxidative E₂DH mRNA respectively in the MDA-MB-231 cell line. PSA (50 ng/ml) induced while higher concentrations reduced reductive

E₂DH mRNA at 24 hours. PSA (200 ng/ml and 400 ng/ml) upregulated oxidative E₂DH mRNA. Both Cathepsin D and PSA had no effect on E₁ level in both cell lines. Cathepsin D (50 ng/ml) increased E₂ levels at 4 and 72 hours whilst reducing E₂ levels at 24 hours compared with 4 hours in the MCF-7 cell line. PSA (200 ng/ml and 400 ng/ml) induced E₂ levels at 4 and 24 hours respectively. Cathepsin D (200 ng/ml) significantly increased E₂ levels at 24 and 72 hours compared with 8 hours in the MDA-MB-231 cell line. Cathepsin D (100 ng/ml) significantly increased E₂ levels at 72 hours versus 4 hours. PSA (200 ng/ml at 8 hours, 200 ng/ml and 400 ng/ml at 72 hours) significantly increased E₂ levels compared with 4 hours. PSA (200 ng/ml and 400 ng/ml) reduced E₂ levels at 4 hours compared with 4 hours.

In conclusion, TGF β ₁, IGF-I and IGF-II may exert synergistic effects on oestrogen metabolism especially in the MCF-7 cell line where they synergistically stimulated the conversion of E₁S to E₁ and E₁ to E₂. Cathepsin D and PSA were unable to activate latent TGF β ₁ and TGF β ₂. Both proteases may regulate the expression of TGF β ₁ mRNA, TGF β ₂ mRNA and E₂ protein in the MCF-7 cell line. They may regulate the expression of TGF β ₁ mRNA, TGF β ₂ mRNA, E₂ protein, reductive and oxidative E₂DH mRNA in the MDA-MB-231 cell line.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia
sebagai memenuhi keperluan untuk ijazah Master Sains

**PERANAN FAKTOR TRANSFORMASI PERTUMBUHAN-BETA, FAKTOR
INSULIN-LIKE PERTUMBUHAN DAN PROTEASE DI KANSER
PAYUDARA MANUSIA**

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Faktor transformasi pertumbuhan-beta ($TGF\beta$) terdapat di sis payudara, tisu payudara yang normal and kanser. Ia memainkan peranan yang penting dalam metabolisme estrogen. Namun demikian, kebanyakan $TGF\beta$ terdapat dalam bentuk laten dan mekanisme di mana laten $TGF\beta$ diaktifkan secara fisiologi masih belum diketahui.

Objektif penyelidikan ini ialah :-

- 1) untuk mengkaji kesan $TGF\beta_1$, IGF-I dan IGF-II terhadap pertumbuhan sel, E_1 STS dan E_2 DH aktiviti di jujukan sel kanser payudara manusia yang bergantung kepada hormon MCF-7 dan tidak bergantung kepada hormon MDA-MB-231.
- 2) untuk mengkaji kesan Cathepsin D dan PSA terhadap $TGF\beta_1$ dan $TGF\beta_2$ mRNA ekspresi, dan paras protein masing-masing di kedua-dua jujukan sel tersebut.

3) untuk mengkaji kesan Cathepsin D dan PSA terhadap Jenis 1 dan Jenis 2 E₂DH mRNA ekspresi, paras E₁ dan E₂ di kedua-dua jujukan sel tersebut.

TGFβ₁, IGF-I dan IGF-II secara persendirian atau dalam kombinasi merencatkan pertumbuhan sel bagi kedua-dua jujukan sel tetapi tiada kesan tambahan atau sinergistik diperhatikan. Rawatan ini merangsang E₁STS aktiviti secara signifikan di jujukan sel MCF-7 kecuali bagi TGFβ₁ sahaja dan TGFβ₁ dan IGF-I secara kombinasi. Hanya TGFβ₁ dan IGF-II merangsang E₁STS aktiviti secara sinergistik di sel MCF-7. Kesemua rawatan ini tidak mempunyai kesan terhadap E₁STS aktiviti di sel MDA-MB-231. Di jujukan sel MCF-7, TGFβ₁ dan IGF-I, IGF-I dan IGF-II, dan TGFβ₁, IGF-I dan IGF-II mempercepatkan reduktif E₂DH secara sinergistik meskipun hanya TGFβ₁, IGF-I dan IGF-II sahaja dapat mempercepatkan oksidatif E₂DH aktiviti secara sinergistik. Tiada kesan sinergistik diperhatikan pada oksidatif and reduktif E₂DH aktiviti di jujukan sel MDA-MB-231.

Cathepsin D (200 ng/ml) meningkatkan TGFβ₁ mRNA pada 72 jam meskipun mengurangkan TGFβ₂ mRNA pada 8 jam bagi 50, 100 dan 200 ng/ml di jujukan sel MCF-7. PSA (50 ng/ml) mengurangkan TGFβ₁ dan TGFβ₂ mRNA pada 72 jam dan 8 jam masing-masing. Cathepsin D (100 ng/ml) meningkatkan TGFβ₂ mRNA pada 24 jam di jujukan sel MDA-MB-231. PSA (50, 200 dan 400 ng/ml) meningkatkan TGFβ₁ mRNA pada 24 jam di sel MDA-MB-231. Kedua-dua Cathepsin D dan PSA tidak dapat mengaktifkan laten TGFβ₁ dan TGFβ₂ di kedua-dua jujukan sel tersebut meskipun terdapat peningkatan TGFβ₂ yang aktif secara signifikan sepanjang tempoh eksperimen. Cathepsin D dan PSA tidak mempunyai kesan ke atas reduktif dan oksidatif E₂DH mRNA di jujukan sel MCF-7. Cathepsin D (50, 100 dan 200

ng/ml) pada 24 jam dan 50 ng/ml pada 4 jam meningkatkan reduktif dan oksidatif E₂DH mRNA masing-masing di jujukan sel MDA-MB-231. PSA (50 ng/ml) meningkatkan meskipun kepekatan tinggi mengurangkan reduktif E₂DH mRNA pada 24 jam. PSA (200 dan 400 ng/ml) meningkatkan oksidatif E₂DH mRNA. Kedua-dua Cathepsin D dan PSA tidak mempunyai kesan terhadap paras E₁ di kedua-dua jujukan sel tersebut. Cathepsin D (50 ng/ml) meninggikan paras E₂ pada 4 dan 72 jam meskipun mengurangkan paras E₂ pada 24 jam berbanding dengan 4 jam di jujukan sel MCF-7. PSA (200 dan 400 ng/ml) meningkatkan paras E₂ pada 4 dan 24 jam masing-masing. Cathepsin D (200 ng/ml) meninggikan paras E₂ secara signifikan pada 24 dan 72 jam berbanding dengan 8 jam di jujukan sel MDA-MB-231. Cathepsin D (100 ng/ml) meninggikan paras E₂ secara signifikan pada 72 jam berbanding dengan 4 jam. PSA (200 ng/ml pada 8 jam, 200 dan 400 ng/ml pada 72 jam) meningkatkan paras E₂ secara signifikan berbanding dengan 4 jam. PSA (200 dan 400 ng/ml) mengurangkan paras E₂ pada 4 jam berbanding dengan 4 jam.

Kesimpulannya, TGF β ₁, IGF-I dan IGF-II boleh memberi kesan sinergistik ke atas estrogen metabolisme terutamanya di jujukan sel MCF-7 di mana mereka merangsang pertukaran E₁S kepada E₁ dan E₁ kepada E₂. Cathepsin D dan PSA tidak dapat mengaktifkan laten TGF β ₁ dan TGF β ₂. Kedua-dua protease mungkin mempengaruhi ekspresi TGF β ₁ mRNA, TGF β ₂ mRNA dan protein E₂ di jujukan sel MCF-7. Mereka juga mempengaruhi ekspresi TGF β ₁ mRNA, TGF β ₂ mRNA, protein E₂, reduktif dan oksidatif E₂DH mRNA di jujukan sel MDA-MB-231.

ACKNOWLEDGEMENTS

No work such as this can be completed without the help and assistance of so many. My appreciation goes first to the funding by the Ministry of Science, Technology and Environment of Malaysia (Grant number: 06-02-04-0053). I would like to thank Dr Patricia KC Lim and the Division of Endocrinology at the Institute Medical Research for allowing us to use their facilities. I also gratefully acknowledge the generosity of Dr Kalanithi Nesaretnam for providing us with the cell lines.

I wholeheartedly thank my supervisors who guided the development of thought in the early stages and helped me in refining my ideas, revising and polishing the content of this thesis. Appreciation also should go to Karin Reimann, Ms Rasha and other colleagues for their invaluable support and guidance. I owe special thanks to my husband and parents for their forbearance and support.

Finally, I recognize that not everyone who has had a hand in putting this together will be properly credited for his / her good works. To those who have helped in some way and I have failed to acknowledge, please forgive this indiscretion and know that in my heart, there is great appreciation for your contribution.

I certify that an Examination Committee met on 9th July 2002 to conduct the final examination of Wong Shew Fung on her Master of Science thesis entitled “Roles of Transforming Growth Factor-Beta, Insulin-like Growth Factors and Proteases in Human Breast Cancer” in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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DECLARATION FORM

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.

WONG SHEW FUNG

Date:

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LIST OF ABBREVIATIONS

$\mu\text{g/L}$	microgram per litre
μl	microlitre
^{14}C	carbon-14
^{35}S	sulphur-35
^3H	tritium
ACTH	adrenocorticotrophic hormone
ATCC	American Type Tissue Culture
bFGF	basic fibroblast growth factor
BLAST	basic local alignment search tool
bp	base pair
cAMP	cyclic adenosine monophosphate
CDK	cyclin dependent kinase
cDNA	complementary deoxyribose nucleic acid
CO_2	carbon dioxide
DEPC	diethylpyrocarbonate
DHEA	dehydroepiandrosterone
DMEM	Dulbecco's Modified Eagle Medium
DMSO	dimethyl sulphoxide
DNA	deoxyribose nucleic acid
dNTP	PCR nucleotide mix
dpm	disintegrations per minute
E_1	oestrone
E_1S	oestrone sulphate
E_1STS	oestrone sulphatase

E ₂	oestradiol
E ₂ DH	oestradiol-17 β hydroxysteroid dehydrogenase
E ₃	oestriol
EDTA	ethylenediamine tetracetic acid
EGF	epidermal growth factor
EGFR	epidermal growth factor receptor
EIA	enzyme immunoassay
ELISA	enzyme-linked immunoabsorbent assay
Endo F	endoglycosidase F
ER	oestrogen receptor
FBS	foetal bovine serum
FGF	fibroblast growth factor
FSH	follicle stimulating hormone
g	gram
GAPDH	glyceraldehyde-3-phosphate dehydrogenase
GC	Guanine - Cytosine
GS	Glycine - Serine
h	hours
HEPES	N-2-hydroxylpiperazine-N'-2-ethanesulphonic acid
His	histidine
HSP	heat shock protein
i.e.	that is
IFN γ	interferon gamma
IGF	insulin-like growth factor
IGFBP	insulin-like growth factor binding protein

IGF-II/M6PR	insulin-like growth factor-II or mannose-6-phosphate receptor
IGF-IIR	insulin-like growth factor-II receptor
IGF-IR	insulin-like growth factor-I receptor
IL	interleukin
IRMA	immunoradiometric assay
K	potassium
kb	kilobase
kDa	kilo Dalton
M	molar
MEM	Minimal Essential Medium
mg/ml	milligram per millilitre
MgCl ₂	magnesium chloride
ml	millilitre
M-MLV	Moloney Murine Leukemia Virus
mRNA	messenger ribonucleic acid
MW	molecular weight
N	normality
Na	sodium
NAD	nicotinamide adenine dinucleotide
NADPH	reduced nicotinamide adenine dinucleotide phosphate
ng/ml	nanogram per millilitre
nm	nanometre
NRK	normal rat kidney
p15	p15 / INK 4B
p21	p21 / WAF 1 / CIP 1

p27	p27 / kip 1
PAI	plasminogen activator inhibitor
PBS	phosphate buffered saline
PCR	polymerase chain reaction
PDGF	platelet-derived growth factor
pg/ml	picogram per millilitre
pM	picomole
pRB	retinoblastoma gene product
PSA	prostate specific antigen
RGD	Arginine - Glycine - Aspartate
rpm	revolutions per minute
RT	reverse transcriptase
RT-PCR	reverse transcriptase polymerase chain reaction
SDS	sodium dodecyl sulphate
sec.	seconds
Ser	serine
SGF	sacroma growth factor
Smad	Sma or Mad related protein
TβR-I	transforming growth factor-beta type I receptor
TβR-II	transforming growth factor-beta type II receptor
TAE	Tris-acetate-EDTA
TGFα	transforming growth factor-alpha
TGFβ	transforming growth factor-beta
TIMP	tissue inhibitor of metalloproteinase
TLC	thin layer chromatography